

**WHAT IS CLAIMED IS:**

1                   1.       A method for separating an intact NP probe from a phosphate  
2 detectable moiety, said method comprising:

3                   a)       providing a sample comprising an intact NP probe with a detectable  
4 moiety attached thereto, whereupon an enzymatic cleavage of said intact NP probe, which  
5 produces a phosphate detectable moiety, said phosphate detectable moiety carries a molecular  
6 charge which is different than the molecular charge of said intact NP probe; and

7                   b)       applying an energy field to said sample, thereby separating said  
8 phosphate detectable moiety from said intact NP probe.

1                   2.       The method according to claim 1, wherein said intact NP probe is a  
2 charge-switch nucleotide phosphate probe having a detectable moiety on a terminal  
3 phosphate.

1                   3.       The method according to claim 2, wherein said charge-switch  
2 nucleotide phosphate is a nucleotide triphosphate (NTP) having a  $\gamma$ -phosphate with a  
3 detectable moiety attached thereto.

1                   4.       The method according to claim 3, wherein said  $\gamma$ -phosphate with a  
2 detectable moiety attached thereto is a  $\gamma$ -phosphate with a fluorophore attached thereto.

1                   5.       The method according to claim 1, wherein said intact NP probe is  
2 incorporated on a primer strand hybridized to a target nucleic acid using a polymerase,  
3 thereby releasing said phosphate detectable moiety.

1                   6.       The method according to claim 1, wherein said polymerase is  
2 immobilized.

1                   7.       The method according to claim 1, wherein said energy field is an  
2 electric field.

1                   8.       The method according to claim 7, wherein said electric field is a first  
2 electric field applied in a transverse direction and a second energy field is applied in an axial  
3 direction.



1                   21.     The method according to claim 18, wherein either said intact NP probe  
2 has a positive molecular charge, or wherein upon cleavage of said phosphate detectable  
3 moiety, said phosphate detectable moiety carries a positive charge relative to said intact NP  
4 probe.

1                   22.     The method according to claim 18, wherein said enzyme is selected  
2 from the group consisting of a DNA polymerase, a DNA dependent RNA polymerase, a  
3 reverse transcriptase, a phosphodiesterase and a phosphatase.

1                   23.     The method according to claim 18, wherein said intact charge-switch  
2 NP probe is a member selected from the group consisting of a nucleotide diphosphate, a  
3 deoxynucleotide triphosphate (dNTP), and a nucleotide triphosphate (NTP).

1                   24.     The method according to claim 23, wherein said deoxynucleotide  
2 triphosphate (dNTP) is a member selected from the group consisting of deoxyadenosine  
3 triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate deoxythymidine  
4 triphosphate and deoxyuridine triphosphate.

1                   25.     The method according to claim 18, wherein said phosphate detectable  
2 moiety is a pyrophosphate with a fluorophore moiety attached thereto.

1                   26.     The method according to claim 25, wherein upon cleavage of said  
2 pyrophosphate fluorophore moiety, said pyrophosphate fluorophore moiety carries a positive  
3 charge relative to said intact NTP probe.

1                   27.     The method according to claim 18, wherein said intact NP probe has a  
2 positive charge.

1                   28.     The method according to claim 18, wherein said intact NP probe has a  
2 negative charge.

1                   29.     An intact charge-switch nucleotide phosphate (NP) probe, wherein,  
2 upon enzymatic cleavage of said intact charge-switch NP probe to produce a phosphate  
3 detectable moiety, said phosphate detectable moiety migrates to an electrode, and intact  
4 charge-switch NP probe migrates to the other electrode.

1                   **30.**    The intact charge-switch NP probe according to claim **29**, wherein  
2 either said intact NP probe has a positive molecular charge, or wherein upon cleavage of said  
3 phosphate detectable moiety, said phosphate detectable moiety carries a molecular positive  
4 charge relative to said intact NP probe.

1                   **31.**    The intact charge-switch NP probe according to claim **29**, wherein said  
2 charge-switch NP probe is a nucleotide triphosphate (NTP); and wherein said phosphate  
3 detectable moiety is a pyrophosphate with a fluorophore moiety attached thereto.

1                   **32.**    The intact charge-switch NP probe according to claim **29**, wherein said  
2 intact NTP probe has a positive charge.

1                   **33.**    The intact charge-switch NP probe according to claim **31**, wherein  
2 upon cleavage of said phosphate detectable moiety as a pyrophosphate fluorophore moiety,  
3 said pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP  
4 probe.

1                   **34.**    The intact charge-switch NP probe according to claim **29**, wherein said  
2 NTP probe is a member selected from the group consisting of a deoxynucleotide triphosphate  
3 (dNTP), and a nucleotide triphosphate (NTP).

1                   **35.**    The intact charge-switch NP probe according to claim **34**, wherein said  
2 NTP probe is a deoxynucleotide triphosphate (dNTP).

1                   **36.**    The intact charge-switch NP probe according to claim **35**, wherein said  
2 deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of  
3 deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate  
4 deoxythymidine triphosphate and deoxyuridine triphosphate.

1                   **37.**    The intact charge-switch NP probe according to claim **34**, wherein  
2 said nucleotide triphosphate (NTP) is a member selected from the group consisting of  
3 adenosine triphosphate, cytosine triphosphate, guanosine triphosphate and uridine  
4 triphosphate.

1                   **38.**    The intact charge-switch NP probe according to claim **31**, wherein  
2 said fluorophore moiety is attached to said terminal phosphate via a linker.

1                   **39.**    The intact charge-switch NP probe according to claim **38**, wherein said  
2 fluorophore linker is an alkylene group having between about 5 to about 12 carbons.

1                   **40.**    The intact charge-switch NP probe according to claim **38**, wherein said  
2 linker carries at least one positive charge.

1                   **41.**    The intact charge-switch NP probe according to claim **38**, wherein said  
2 linker carries at least two positive charges.

1                   **42.**    The intact charge-switch NP probe according to claim **29**, wherein at  
2 least one of the phosphate moieties of said nucleotide phosphate probe has an ionized oxygen  
3 atom with a counter-cation associated therewith.

1                   **43.**    The intact charge-switch NP probe according to claim **29**, wherein said  
2 counter-cation is a metal ion.

1                   **44.**    The intact charge-switch NP probe according to claim **43**, wherein said  
2 metal ion is selected from the group consisting of  $Mg^{++}$ ,  $Mn^{++}$ ,  $K^{+}$  and  $Na^{+}$ .

1                   **45.**    A method for sequencing a nucleic acid, said method comprising:  
2 providing a target nucleic acid, a primer strand, a polymerase, and a plurality  
3 of NP probes;  
4 mixing said target nucleic acid, said sequencing primer, said polymerase, said  
5 plurality of NP probes in a flowcell under conditions permitting target dependent  
6 polymerization of said plurality of NP probes, thereby providing a polymerization product;  
7 and  
8 separating the polymerization products by an energy field in said flowcell to  
9 provide a sequence of said target nucleic acid.

1                   **46.**    The method according to claim **45**, wherein the polymerization of said  
2 plurality NP probes produces a plurality of phosphate detectable moieties.

1                   **47.**    The method according to claim **45**, wherein said plurality of NP probes  
2 are incorporated on said primer strand hybridized to said target nucleic acid using said  
3 polymerase, thereby releasing a  $\gamma$ -phosphate with a detectable moiety attached thereto.

